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Compositional features and biological activities of wild and commercial *Moringa oleifera* leaves from Guinea-Bissau

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ABSTRACT

Moringa oleifera Lam. is a nutraceutical edible plant used to fight malnutrition in developing countries. This investigation describes the nutritional, chemical and bioactive assets of wild and commercially acquired *M. oleifera* leaves from Guinea-Bissau. Both samples presented significant differences in the contents of almost all the analysed parameters. Carbohydrates and proteins were the major constituents of the leaves, which also showed an interesting profile of tocopherols, organic acids, unsaturated fatty acids, and phenolic compounds. The infused extract of the commercial leaf sample was more effective than other preparations against lipid peroxidation and oxidative haemolysis. In turn, higher antibacterial activity was achieved with hydroethanolic and infused extracts of the wild sample, which extracts also displayed superior antifungal activity. Overall, the commercially acquired *M. oleifera* leaves stand out with better quality profiles than the wild collected, which aroused interest in studying the processing methods used locally to process this functional food.

1. Introduction

In addition to their many medicinal properties, wild edible plants have been used worldwide as a significant element of human nutrition (Pinela et al., 2017). Recently, plant-based functional foods and nutraceuticals, as well as their potential contribution to human health and well-being, have stood out in the field of scientific research (Iwu, 2017; Muyonga et al., 2020). Mostly in developing countries, the food security is threatened given the exponential population growth, climate changes, natural disasters, and different types of conflicts that undermine the sustainability of agricultural and food systems (FAO, 2017). In West Africa, where malnutrition problems persist, these trends represent a series of challenges to food and agriculture (Leakey, 2017). Thus, the use of plant-based foods with nutraceutical properties, obtained from wild or easily cultivated species, presents itself as a good approach to face these challenges (Gul et al., 2016).

Moringa oleifera Lam. (Fam. Moringaceae) is a fast-growing tree, native to India and Pakistan, which was introduced in the tropics and sub-tropics around the world, becoming natural in several African countries due to its high drought tolerance. and ability to thrive in warm and semi-arid regions (Bancessi et al., 2020). This multifunctional plant is commonly used not only as a vegetable, but also as a traditional remedy, as it contains a valuable nutritive profile, with considerable amounts of proteins, carbohydrates, minerals, and vitamins, as well as several medicinal properties. In fact, almost all portions of this plant is suitable to be exploited for food, agricultural, medicinal, and industrial determinations, being an economically valued crop, especially in the developing countries (Zungu et al., 2020).

The bark, root, leaves, and flowers of *M. oleifera* are used in several countries to prepare remedies used in folk medicine to handle with skin diseases, anaemia, cholera, and further illnesses. Particularly, fresh leaves are commonly consumed after cooking, or prepared in soup or

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salads, being also employed as a food preservative to enhance the shelf-life of food, such as meat, by minimizing the oxidation processes. In turn, dried leaves can be processed into powder, thus being readily available for adding to foods, such as smoothies and a variety of beverages and other products (Olusanya et al., 2020). *M. oleifera* leaves are also used as a good alternative to fight malnutrition, especially in children, young individuals, and pregnant women, mainly due to their high content of protein and dietary fibre, and their capacity to improve the quality of breastfeeding milk. Besides their rich nutritional composition, *M. oleifera* leaves have also been reported to contain large amounts of bioactive phytochemicals, particularly phenolic compounds, which are associated with the different beneficial effects on human health (Zungu et al., 2020).

This deciduous tree has the potential to improve food security, human nutrition, and promote rural progress and development. To maximize the benefits of domesticated plants with socio-economic and technological potential for the world population, it is important to recognize how they differ in compositional terms from their wild form, where the last represent a storehouse of genetic diversity (Brozynska et al., 2016; Migicovsky & Myles, 2017).

In this sense, this study was carried out to provide a detailed characterization of the nutritional profile and chemical configuration of *M. oleifera* leaves collected *in natura* and processed locally (Guinea-Bissau), as well as the *in vitro* antioxidant and antimicrobial activities of their hydroethanolic and aqueous extracts (prepared according to traditional practices).

2. Material and methods

2.1. Specimen and samples preparation

Wild leaf samples of *M. oleifera* (Fig. 1a and b) were collected in April 2019 in Bolama, Bolama Island (11° 34′38″N 15° 28′48″W), Guinea-Bissau, and shade-dried, processed into a fine powder and stowed in taped up plastic bags. Commercial leaf samples, already processed (Fig. 1c), were acquired in May 2019 in the Caritas Community Pharmacy in Bissau. These last samples were produced and processed at Contuboel, Eastern country (12° 22′50″N 14° 33′58″W) trough a partnership between Caritas and a group of local women. Both samples were stored at −20 °C until analysis.

In both sites, Bolama and Contuboel, *M. oleifera* is grown under rainfed conditions on Ferralsols as living fence or near the houses in the villages. However, there are some differences between these sites concerning the climate. Bolama, located at southern coastal region, receives an annual rainfall of c. 2300 mm, while in Contuboel, in inner eastern country, the annual rainfall is c. 1500 mm. Also, the coastal influence at Bolama attenuates temperature differences and provides a less dry environment in the dry season (Catarino et al., 2008).

2.2. Nutritional value and energy determination

Macronutrients amount (protein, fat, and ash; g/100 g) were assessed following the AOAC official procedures of food analysis (AOAC International, 2016). The macro-Kjeldahl technique was utilized to estimate the crude protein quantity ($N \times 6.25$); crude fat was determined through extraction with petroleum ether in a Soxhlet apparatus; the ash totals were measured by incineration at 550 ± 10 °C. The total

Fig. 1. *Moringa oleifera* leaf samples wild-harvested in Bolama (a and b) and commercially acquired, produced in Contuboel (c).

carbohydrate sum (g/100 g of dried weight (dw)) was determined by difference. The energy (kcal/100 g dw) was calculated following the equation: 4 × (g protein + g carbohydrates) + 9 × (g fat).

2.3. Analysis of free sugars and organic acids

The free sugars configuration was determined by high-performance liquid chromatography (HPLC) coupled to a refraction index (RI) detector, by means of the internal standard (IS, melezitose; Sigma-Aldrich, St. Louis, MO, USA) method, as before defined (Spréa et al., 2020). The identification was performed by comparing the retention times of the authentic standards with those of the samples, whereas quantification was achieved by the IS method, with calibration curves built up with the standards. The results were stated in g per 100 g of dw.

The organic acids profile was identifyed by ultra-fast liquid chromatography (UFLC; Shimadzu 20A series, Kyoto, Japan) through a procedure previous labelled and optimized by the authors (Pereira et al., 2013). Detection was done in a photo-diode array detector (PDA), using 215 nm and 280 nm as preferable wavelengths. Quantification was completed by likening the peak area of the samples with calibration curves made with commercial standards. The organic acids quantity was expressed in g per 100 g of dw.

2.4. Analysis of fatty acids and tocopherols

The fatty acid methyl esters (FAME) profile was achieved after *trans*-esterification of the lipid fraction attained by Soxhlet extraction (Spréa et al., 2020), followed by gas-liquid chromatography with flame ionization detection, using a YOUNG IN Crhomass 6500 GC System apparatus equipped with a *split/splitless* injector, a flame ionization detector (FID), and a Zebron-Fame column. Identification and quantification were completed by associating the relative retention times of the FAME peaks of the samples, with those of the standard (47885-U; Sigma-Aldrich, St. Louis, MO, USA). The Clarity DataApex 4.0 Software (Prague, Czech Republic) was utilized for data handing. The results were expressed in relative percentage (%) of each detected fatty acid.

Tocopherols were characterized following an analytical procedure before described by the authors (Spréa et al., 2020). An HPLC system coupled to a fluorescence detector (FP-2020; Jasco) programed for excitation at 290 nm and emission at 330 nm was utilized. The isoforms identification was attained by chromatographic comparison with authentic standards and the quantification was founded on the fluorescence signal response of each standard, using the IS (tocol (50 mg/mL); Matreya, Pleasant Gap, PA, USA) method and calibration curves built with commercial standards. The results were expressed in mg per 100 g of dw.

2.5. Polyphenolic profile characterization

2.5.1. Preparation of hydroethanolic and aqueous extracts

The plant material was used to make hydroethanolic, infusion, and decoction extracts. The first was made by stirring the plant material (~2.5 g) with 30 mL of ethanol/water (80:20, *v/v*) at 25 °C for 1 h, and filtered through Whatman No. 4 paper. The deposit was then re-extracted with an extra 30 mL of the hydroalcoholic mixture. The joint extracts were concentrated at 40 °C under reduced pressure (rotary evaporator Büchi R-210, Flawil, Switzerland) and further lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA). For infusion extracts, 100 mL of freshly boiled distilled water (heating plate, VELP scientific) were added to the plant material (~2.5 g), left aside for 5 min and subsequently filtered through Whatman filter paper No 4. The resultant extracts were frozen and lyophilized. Decoctions were done by adding 200 mL of distilled water to the plant material (~2.5 g), and boiled for 5 min. Next, the mixtures were left to rest for 5 min and then filtered through Whatman No. 4 paper. The obtained decoctions were frozen and lyophilized.

2.5.2. Analysis of phenolic compounds

Phenolic compounds were investigated in the hydroethanolic, infused and decocted extracts, which were redissolved in ethanol/water (80:20, *v/v*) and water, respectively, to a final concentration of 10 mg/mL, and filtered thought 0.22-μm disposable filter disks. The analysis was made in a HPLC system (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA) coupled with a diode-array detector (DAD, using 280 nm and 370 nm as preferred wavelengths) and a Linear Ion Trap (LTQ XL) mass spectrometer (MS, Thermo Finnigan, San Jose, CA, USA) prepared with an electrospray ionization (ESI) source. The separation was made in a Waters Spherisorb S3 ODS-2 C18 column (3 μm, 4.6 mm × 150 mm; Waters, Milford, MA, USA), and the elution solvents, working in the gradient, were 0.1 % formic acid in water and acetonitrile. The equipment and operating conditions were earlier optimized by Bessada et al. (2016). Identification was performed based on chromatographic behavior, spectra, and UV–Vis masses, by comparison with standard compounds or data earlier described in the literature, using the Xcalibur® software (ThermoFinnigan, San Jose, CA, USA). Quantitative analysis of the detected compounds was achieved employing calibration curves based on the UV signal of the standard compounds. When commercial standards were not available, the calibration curves of the most similar standards were used. The phenolic standards were acquired from Extrasynthèse, Genay, France. The results were expressed as mg per g of extract.

2.6. Antioxidant activity evaluation

2.6.1. Thiobarbituric acid reactive substances (TBARS) formation inhibition capacity

The lyophilized extracts were re-dissolved in water and subjected to dilutions from 2.5 mg/mL to 0.0390 mg/mL. The lipid peroxidation inhibition in porcine brain cell homogenates was evaluated by the reduction in TBARS; the colour strength of malondialdehyde–thiobarbituric acid (MDA–TBA) was measured at 532 nm; the inhibition ratio (%) was considered using the formula: $[(A - B)/A] \times 100$ %, where A and B correspond to the absorbance of the control and extract sample, respectively (Spréa et al., 2020). The results were expressed in IC_{50} values (μg/mL, sample concentration providing 50 % of antioxidant activity). Trolox (Sigma-Aldrich, St. Louis, MO, USA) was used as positive control.

2.6.2. Oxidative haemolysis inhibition (OxHLIA) capacity

The antihaemolytic activity of the lyophilized extracts was evaluated by the oxidative haemolysis inhibition assay (OxHLIA) using red blood cells (RBC) isolated from the blood of healthy sheeps, as described by the authors (Silva de Sá et al., 2019n) resultant from the half haemolysis time (Ht_{50} values) gotten from the haemolytic curves of each extract sample concentration minus the Ht_{50} value of the PBS control, were associated to the corresponding extract concentration to obtained IC_{50} values (μg/mL), which were calculated for 60 and 120 min, *i.e.*, extract concentration required to protect 50 % of the erythrocyte population from the haemolytic action of 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH; Sigma-Aldrich, St. Louis, MO, USA) for 60 and 120 min. Trolox was used as a positive control.

2.7. Antimicrobial activity evaluation

Bacillus cereus (food isolate), *Staphylococcus aureus* (American Type Culture Collection, Manassas, VA, USA, ATCC 6538), *Listeria monocytogenes* (National Collection of Type Cultures, London, UK, NCTC 7973), *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 35030), and *Salmonella typhimurium* (ATCC 13311) were selected to test the antibacterial activity of the extracts. For antifungal activity, six micromycetes were used, namely *Aspergillus fumigatus* (ATCC 9197), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus niger* (ATCC 6275), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC

9112), and *Penicillium verrucosum* var. *cyclopium* (food isolate). The microdilution method was performed as previously described (Soković et al., 2010). The results were presented as the concentrations that resulted in complete inhibition of the bacterial growth (MIC, minimal inhibition concentration), through the colorimetric microbial viability assay, as well as MBC and MFC values (minimal bactericidal concentration and minimal fungicidal concentration, respectively). The used positive controls were streptomycin, ampicillin, ketoconazole, and bifonazole (Sigma-Aldrich, St. Louis, MO, USA), whereas the negative control was 5 % dimethyl sulfoxide (DMSO).

2.8. Statistical analysis

The experiments were carried out in triplicate and the results were expressed as mean ± standard deviation. the SPSS Statistics Software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) was used to assess significant differences among the two samples by applying a two-tailed paired Student's *t*-test. For phenolic compounds, differences among samples were assessed applying one-way analysis of variance (ANOVA). The fulfilment of the ANOVA requirements was tested through the Shapiro Wilk's and the Levene's tests to assess the normality and variance homogeneity of the data, respectively. Results were compared using a Tukey's HSD test. Significant differences were considered when the *p*-value was lower than 0.05.

3. Results and discussion

3.1. Nutritional composition of edible M. oleifera leaves

The results obtained for the *M. oleifera* leaf proximate composition are presented in Table 1. Carbohydrates were the main constituents found in wild and commercial leaf samples (51.5 and 50.8 g/100 g dw, respectively), whose levels did not differ significantly ($p > 0.05$) between samples.

Table 1
Nutritional value, free sugars and organic acids composition of *M. oleifera* leaves.

	Wild sample	Commercial sample	*p*-value
Nutritional value (g/100 g dw)			
Crude fat	8.7 ± 0.1	8.0 ± 0.4	0.016
Proteins	27.4 ± 0.3	32.6 ± 0.9	<0.001
Ash	12.4 ± 0.1	8.63 ± 0.09	<0.001
Total carbohydrates	51.5 ± 0.4	50.8 ± 0.5	0.090
Energy (kcal/100 g dw)	393.7 ± 0.2	406 ± 2	<0.001
Free sugars (g/100 g dw)			
Fructose	0.94 ± 0.01	0.81 ± 0.03	0.001
Glucose	0.41 ± 0.01	0.57 ± 0.03	<0.001
Sucrose	1.97 ± 0.02	5.33 ± 0.09	<0.001
Trehalose	0.84 ± 0.01	1.20 ± 0.05	<0.001
Total sugars	4.16 ± 0.02	7.9 ± 0.2	<0.001
Organic acids (g/100 g dw)			
Oxalic acid	1.83 ± 0.01	1.97 ± 0.01	<0.001
Malic acid	3.95 ± 0.04	4.08 ± 0.01	0.001
Ascorbic acid	0.007 ± 0.001	0.008 ± 0.001	0.013
Citric acid	2.60 ± 0.01	2.91 ± 0.04	<0.001
Fumaric acid	tr	tr	–
Total organic acids	8.38 ± 0.04	8.97 ± 0.06	<0.001

Results are expressed mean ± SD ($n = 3$); tr: traces; Free sugars calibration curves: fructose (y = 1.04x, $R^2 = 0.999$; LOD = 0.05 mg/mL, LOQ = 0.18 mg/mL), glucose (y = 0.935x, $R^2 = 0.999$; LOD = 0.08 mg/mL; LOQ = 0.25 mg/mL); sucrose (y = 0.977x, $R^2 = 0.999$; LOD = 0.06 mg/mL, LOQ = 0.21 mg/mL) and trehalose (y = 0.991x, $R^2 = 0.999$; LOD = 0.07 mg/mL, LOQ = 0.24 mg/mL). Organic acids calibration curves: oxalic acid (y = 9E + 10^6x + 459.731; $R^2 = 0.994$; LOD = 12.55 μg/mL; LOQ = 41.82 μg/mL); malic acid (y = 912.441x + 92.665; $R^2 = 0.999$; LOD = 35.76 μg/mL; LOQ = 119.18 μg/mL); ascorbic acid (y = 7E + 07x + 60.489; $R^2 = 0.999$; LOD = 367 μg/mL; LOQ = 1222 μg/mL); citric acid (y = 1E + 10^6x + 45.682; $R^2 = 1$; LOD = 10.47 μg/mL; LOQ = 34.91 μg/mL) and fumaric acid (y = 2E + 08x + 1E + 06; $R^2 = 1$; LOD = 0.08 μg/mL; LOQ = 0.26 μg/mL).

These values are slightly lower than those (56.6 g/100 g dw) reported by Ziani et al. (2019), which investigate the nutritional value of *M. oleifera* cultivated in desert areas of South Algeria. Contrary to the observed for carbohydrates, statistically significant differences ($p < 0.05$) were found for the nutritional value constituents analysed in the wild and commercial *M. oleifera* leaf samples (Table 1). The amount of proteins ranged from 27.4 g/100 g dw in the samples collected in the wild to 32.6 g/100 g dw in the commercially acquired one. In a previous study, Chodur et al. (2018) analysed domesticated and wild samples of *M. oleifera* and recorded a higher protein content in the domesticated plants than in the wild collected ones (30.2 and 26.3 g/100 g, respectively), although these values did not differ significantly. A higher ash content was registered in the wild sample than in the commercial one (12.4 and 8.63 g/100 g dw). Valdez-Solana et al. (2015) reported an appreciable amount (~11 g/100 g) of ashes in the leaves of two Mexican *M. oleifera* cultivars, comprised within the values of this study. These results translate the high mineral content that can be found in *M. oleifera* edible leaves. In turn, crude fat was the macronutrient found in lower amounts, with values ranging from 8.0 to 8.7 g/100 g dw in commercial and wild samples, respectively. A low amount of fat (2.82 g/100 g) was also found by Ilyas et al. (2015), which demonstrates that *M. oleifera* leaves can be suitable foods for low-fat diets, with an energy contribution ranging from 394 to 406 kcal/100 g dw (Table 1).

Table 1 presents the free sugars composition of *M. oleifera* leaves. Fructose, glucose, sucrose, and trehalose were detected in both samples and sucrose was the major free sugar, with levels varying significantly ($p < 0.001$) from 1.97 g/100 g dw in the wild sample to 5.33 g/100 g dw in the commercial one. In a previous study, Al Juhaimi et al., 2017 reported small amounts of raffinose and stachyose in *M. oleifera* leaves, but did not detect trehalose. The authors quantified glucose as the most abundant free sugar in their samples (6.65 g/100 g), followed by fructose (3.96 g/100 g), a result that differs from this study where sucrose was the most abundant free sugar. All these variances might be owed to edaphoclimatic influences, such as temperature variations and soil composition, as well as some biotic conditions that can influence the synthesis of sugars.

Concerning organic acids, oxalic, malic, ascorbic, and citric acids were identified in the studied leaf samples (Table 1). Malic acid, a dicarboxylic acid that contributes to a sour taste and used as a food additive was the most abundant (~4 g/100 g dw). The levels of each identified organic acid differed significantly ($p \leq 0.05$) between samples. The observed differences may be related to the maturity of the leaves at harvest, drying and storage practices and the processing conditions applied to the samples (Dias et al., 2016). Previously, Ziani et al. (2019) identified three organic acids in *M. oleifera* leaves, namely oxalic, malic, and ascorbic acids; malic acid was also detected in higher amounts (2.5 g/100g dw), followed by oxalic and ascorbic acids. The authors did not identify citric acid (a natural food additive), although it was found in considerable amounts in our samples, a result that supports the use of this plant as a traditional food preservative.

The fatty acids profile results are presented in Table 2. A total of 21 compounds were identified in the analysed *M. oleifera* leaves. α-Linolenic acid (C18:3n3) was the most abundant, followed by palmitic (C16:0) and linoleic (C18:2n6) acids. C16:0 was the only fatty acid that did not show significant differences ($p > 0.05$) between samples. Considering their classification, polyunsaturated fatty acids (PUFA) represented the higher percentage of all fatty acids, with values ranging from 58.5 to 58.8%, followed by saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). Both leaf samples presented significant differences in the contents of SFA and MUFA. Castillo-López et al. (2017) in a study that aimed to characterize two *M. oleifera* leaf variants that grow in Sinaloa, Mexico, identified 14 fatty acids, where the α-linolenic acid was found in higher amounts (ranging from 62.72 to 66.79%), followed by palmitic and linoleic acids, being PUFA the

Table 2
Fatty acids and tocopherols composition of *M. oleifera* leaves.

	Wild sample	Commercial sample	*p*-value
Fatty acids (relative %)			
C6:0	0.31 ± 0.01	0.31 ± 0.02	0.584
C8:0	0.08 ± 0.01	0.096 ± 0.005	0.003
C10:0	0.143 ± 0.003	0.199 ± 0.006	<0.001
C11:0	0.26 ± 0.01	0.299 ± 0.009	0.004
C12:0	0.144 ± 0.008	0.326 ± 0.003	<0.001
C14:0	1.34 ± 0.03	1.8 ± 0.1	0.001
C15:0	0.190 ± 0.001	0.149 ± 0.007	<0.001
C16:0	17.33 ± 0.01	17.6 ± 0.4	0.674
C16:1	1.75 ± 0.06	1.57 ± 0.02	0.003
C17:0	0.30 ± 0.01	0.29 ± 0.01	0.381
C18:0	4.2 ± 0.1	3.3 ± 0.2	<0.001
C18:1n9c	4.04 ± 0.05	5.7 ± 0.4	<0.001
C18:2n6c	8.2 ± 0.1	10.1 ± 0.2	<0.001
C18:3n3	50.1 ± 0.2	48.1 ± 0.1	<0.001
C20:0	1.11 ± 0.01	1.31 ± 0.03	<0.001
C20:1	0.42 ± 0.01	0.132 ± 0.002	<0.001
C21:0	0.168 ± 0.006	0.17 ± 0.01	0.515
C22:0	4.1 ± 0.1	4.0 ± 0.3	0.359
C22:2	0.48 ± 0.03	0.246 ± 0.005	<0.001
C23:0	0.40 ± 0.01	0.28 ± 0.01	<0.001
C24:0	4.73 ± 0.09	4.07 ± 0.09	<0.001
SFA	35.0 ± 0.3	34.1 ± 0.5	0.019
MUFA	6.21 ± 0.02	7.4 ± 0.4	0.001
PUFA	58.8 ± 0.3	58.5 ± 0.1	0.75
Tocopherols (mg/100 g dw)			
α-Tocopherol	58.79 ± 0.03	68.03 ± 0.05	<0.001
β-Tocopherol	3.23 ± 0.04	2.41 ± 0.06	<0.001
δ-Tocopherol	10.85 ± 0.07	6.63 ± 0.01	<0.001
Total tocopherols	72.87 ± 0.06	77.1 ± 0.1	<0.001

Results are expressed mean ± SD ($n = 3$); caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), undecanoic acid (C11:0), lauric acid (C12:0), myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1n9c), linoleic acid (C18:2n6c), α-linolenic acid (C18:3n3), arachidic acid (C20:0), *cis*-11-eicosenoic acid (C20:1), eicosenoic acid (C21:0), behenic acid (C22:0), *cis*-13,16-docosadienoic acid (C22:2), tricosanoic acid (C23:0), and lignoceric acid (C24:0); SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; tr: traces. Tocopherols calibration curves: α-tocopherol ($y = 1.295 \times$; $R^2 = 0.991$; LOD: 18.06 ng/mL, LOQ: 60.20 ng/mL); β-tocopherol ($y = 0.396 \times$; $R^2 = 0.992$; LOD: 25.82 ng/mL, LOQ: 86.07 ng/mL; γ-tocopherol ($y = 0.567 \times$; $R^2 = 0.991$; LOD: 14.79 ng/mL, LOQ: 49.32 ng/mL); δ-tocopherol ($y = 0.678 \times$; $R^2 = 0.992$; LOD: 20.09 ng/mL, LOQ: 66.95 ng/mL).

detached class of fatty acids in both samples. According to various studies, the consumption of PUFA is responsible for decreasing the levels of total and low-density lipoprotein (LDL) cholesterol, playing a cardioprotective role in human organisms (Ander et al., 2003).

Regarding tocopherols, the isoforms α-, β- and δ-tocopherol were identified in both wild and commercial leaf samples (Table 2) and α-tocopherol was the main isoform (with 58.79 and 68.03 mg/100 g dw, respectively), followed by δ-tocopherol, and lastly β-tocopherol. Therefore, a 100 g serving of *M. oleifera* leaves provides more vitamin E than the recommended dietary allowances (15 mg/day) for adult individuals. It was also observed that the origin of the plant material caused significant differences ($p < 0.001$) in the content of these lipophilic antioxidants. A study of Ziani et al. (2019) describes the four tocopherol isoforms in amounts different than those found in this study; the authors also reported α-tocopherol as the main isoform (quantified in lower amounts of 4.0 mg/100 g dw), followed by γ-tocopherol (with just 0.41 mg/100 g dw). These compositional differences may be due to the edaphoclimatic conditions of the growing sites, abiotic factors, and the processing methods used in samples preparation.

3.2. Polyphenolic composition of *M. oleifera* leaf extracts

The data attained regarding the chromatographic features (retention time, λ_{max}, pseudomolecular ions, and main MS^2 fragments), as well as the tentative identification of the phenolic compounds found in each hydroethanolic, infused and decocted extract of *M. oleifera* leaves are shown in Table 3. Twenty-five compounds were identified, 6 of which were phenolic acids and 19 derived from glycosylated flavonols. Regarding the phenolic acids and considering the above parameters, the

Table 3
Phenolic compounds identified in hydroethanolic, infused and decocted *M. oleifera* leaf extracts. It is presented the retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), and mass spectral data.

Peak	Rt (min)	λ_{max} (nm)	[M-H] (*m/z*)	MS^2 (*m/z*)	Tentative identification
1	4.15	324	341	179(100), 161 (3), 135(40)	Caffeic acid hexoside
2	4.69	324	353	191(100), 179 (47), 173(5), 161 (3), 135(10)	3-*O*-Caffeoylquinic acid
3	4.88	324	353	191(20), 179 (55), 173(100), 161(5), 135(8)	4-*O*-Caffeoylquinic acid
4	6.25	280	337	191(8), 173(5), 163(100), 155 (5), 137(3), 119 (5)	3-*p*-Coumaroylquinic acid
5	6.40	324	353	191(100), 179 (80), 173(24), 161(5), 135(10)	*cis*-5-*O*-Caffeoylquinic acid
6	7.08	324	353	191(100), 179 (80), 173(42), 161(5), 135(12)	*trans*-5-*O*-Caffeoylquinic acid
7	9.86	338	593	473(100), 383 (15), 353(25)	Apigenin-6,8-di-*C*-hexoside
8	10.29	338	593	473(100), 383 (15), 353(25)	Apigenin 8-*C*-glucoside 6″-*O*-glucoside
9	11.39	338	593	473(100), 383 (15), 353(25)	Apigenin 6-*C*-glucoside 7-*O*-glucoside
10	13.47	338	593	473(100), 383 (15), 353(25)	Apigenin 6,8-*C*-di-β-*D*-glucopyranoside
11	14.86	327	447	301(100)	Quercetin-3-*O*-rhamnoside
12	16.43	352	431	341(27), 311 (100)	Apigenin-8-*C*-glucoside
13	17.67	352	609	301(100)	Quercetin-3-*O*-rutinoside
14	18.24	352	609	301(100)	Quercetin-4′-*O*-rhamnoside-7-*O*-hexoside
15	18.86	352	609	301(100)	Quercetin-3-*O*-rhamnoside-7-*O*-hexoside
16	19.02	352	463	301(100)	Quercetin 3-*O*-glucoside
17	19.77	350	505	301(100)	Quercetin-3-*O*-(6″-acetyl)-glucoside
18	20.11	350	549	505(10), 463 (27), 301(100)	Quercetin-3-*O*-(6″-malonyl-glucoside)
19	21.00	350	549	505(10), 463 (27), 301(100)	Quercetin 7-*O*-malonylglucoside
20	22.04	350	549	505(10), 463 (27), 301(100)	Quercetin-*O*-malonyl-hexoside
21	23.4	350	549	505(10), 463 (27), 301(100)	Quercetin 3-*O*-malonylglucoside
22	24.07	350	623	315(100)	Isorhamnetin-3-*O*-rutinoside
23	24.59	343	533	285(100)	Kaempferol-3-*O*-(6″-malonyl-glucoside)
24	25.33	349	519	315(100)	Isorhamnetin-3-O-(6″-acetyl)-glucoside
25	25.88	350	563	315(100)	Isorhamnetin-3-O-(6″-malonyl-glucoside)

compounds were tentatively identified as caffeic acid hexoside (peak **1**; λ_{max}, 324 nm; $[M-H]^-$ at *m/z* 341), 3-*O*- and 4-*O*-caffeoylquinic acids (peaks **2** and **3**; λ^{max}, 324 nm; $[M-H]^-$ at *m/z* 353, respectively), 3-*p*-coumaroylquinic acid (peak **4**; λ_{max}, 280 nm; $[M-H]^-$ at *m/z* 337), and finally *cis* and *trans* 5-*O*-caffeoylquinic acids (peak **5** and **6**, respectively; λ_{max}, 324 nm; $[M-H]^-$ at *m/z* 353). Chlorogenic acids are a large family of polyphenols that function as antioxidants and as dietary neuro-signalling compounds, improving lipid metabolism and promoting weight loss by reducing the synthesis of visceral fat, cholesterol, and fatty acids (Nakatani et al., 2000). These phenolic acids are composed of quinic acid linked to *trans*-cinnamic acids, such as caffeic acid, via an ester bond.

Although the most common chlorogenic acid is 5-*O*-caffeoylquinic, other isomers may be present in the same plant, such as 3 and 4-*O*-caffeoylquinic acids (Braham et al., 2020a). Since *M. oleifera* has been widely studied through the last decades, the compounds found in our study have previously been identified in the foliar parts of this species from Brazil, namely the caffeic acid (Oldoni et al., 2019), and from South Algeria decocted and hydroethanolic extracts (peaks 2, 3 and 4) by Ziani et al. (2019). However, our study also reveals the presence of *cis* and *trans* 5-*O*-caffeoylquinic acid compounds, while Braham et al. (2020b) only used this chlorogenic acid as a pattern (5-*O*-caffeoylquinic acid) to identify the respective isomers. In fact, and contrary to our study, Castro-López et al. (2017) reported that the only chlorogenic acid present in *M. oleifera* leaf extracts obtained by decoction is the 3-*O*-caffeoylquinic acid.

The group of flavonoids proved to be the most abundant in the prepared *M. oleifera* leaf extracts, among which stands out the quercetin derivatives, present in greater number than the other identified aglycone flavonoids, followed by apigenin, isorhamnetin and kaempferol derivatives in glycosidic and isomeric forms. Thus, our experimental conditions allow us to identify quercetin-3-*O*-rhamnoside (peak **11**; $[M-H]^-$ at *m/z* 447), quercetin-3-*O*-rutinoside (peak **13**; $[M-H]^-$ at *m/z* 609), quercetin 3-*O*-glucoside (peak **16**; [M-H] at *m/z* 463), and quercetin-3-*O*-(6″-malonyl-glucoside) (peak **18**; [M-H] at *m/z* 549), already previously identified in *M. oleifera* leaves from Southern Italy (Rocchetti et al., 2020) and Ghana (Amaglo et al., 2010a), respectively, and the last two compounds by Bennett et al. (2003) from different locations of Africa. Peaks **14**/**15**, presented both a pseudomolecular ion $[M-H]^-$ at *m/z* 609, and MS^2 fragment at *m/z* 301, allowing its tentative identification as quercetin-4-*O*-rhamnoside-7-*O*-hexoside and quercetin-3-*O*--rhamnoside-7-*O*-hexoside, respectively, firstly identified in *M. oleifera* leaves in our study.

Given this assumption, its tentative identification was achieved following the earlier described by Carocho et al. (2014) in *Castanea sativa* Mill. flower extracts. Likewise, peak **17** ($[M-H]^-$ at *m/z* 505) and peaks **19**–**21** (([M-H] at *m/z* 549) were tentatively identified as quercetin-3-*O*-(6″-acetyl)-glucoside (Jang et al., 2018), quercetin 7-*O*-malonylglucoside (Guimarães et al., 2014), quercetin-*O*-malonyl-hexoside (Bessada et al., 2016), and quercetin 3-*O*-malonylglucoside (Lin et al., 2020), as described by the authors. A total of 6 quercetin derivatives were, to the best of our knowledge, identified for the first time in *M. oleifera* leaf extracts, probably owing to specific environmental characteristics and geographic location in which this species grows (Ma et al., 2020). Quercetin is described in the literature for its high antioxidant capacity, given its high ability to eliminate reactive oxygen species, and for its antitumor properties, since it has the ability to modulate cell apoptosis and the migration and growth of tumour cells through the various signalling pathways involved (Lesjak et al., 2018). Apigenin-derived compounds appear as the second largest group of flavonoids herein identified, with peaks **7**–**10** ($[M-H]^-$ at *m/z* 593) tentatively identified as apigenin-6,8-di-*C*-hexoside, apigenin 8-*C*-glucoside-6″-*O*-glucoside, apigenin-6-*C*-glucoside-7-*O*-glucoside, and apigenin-6,8-*C*-di-β-*D*-glucopyranoside, respectively, following the before defined by Ferreres et al. (2007), and once again herein identified for the first time in *M. oleifera* species. On the other hand, peak **12** ($[M-H]^-$ at *m/z* 431), tentatively identified as apigenin-8-*C*-glucoside, was previously reported by Karthivashan et al. (2013) in extracts with different hydroethanolic gradients of *M. oleifera* leaves from Malaysia.

Three isorhamnetin derivatives were also identified in our study, namely isorhamnetin-3-*O*-rutinoside (peak **22**; $[M-H]^-$ at *m/z* 623), isorhamnetin-3-*O*-(6″-acetyl)-glucoside (peak **24**; $[M-H]^-$ at *m/z* 519), and isorhamnetin-3-*O*-(6''-malonyl)-glucoside (peak **25**; $[M-H]^-$ at *m/z*) 563, being the first two identified in *M. oleifera* leaf samples from South Africa by Makita et al. (2016) and the last compound (peak 25) by Amaglo et al. (2010) in samples from Ghana. The only kaempferol derivate tentatively identified in our study, namely kaempferol-3-*O*-(6″-malonyl-glucoside) (peak **23**; $[M-H]^-$ at *m/z* 533), was also identified in the same samples of *M. oleifera* leaves from Ghana (Amaglo et al., 2010b).

Table 4 presents the quantitative data of the phenolic compounds tentatively identified in *M. oleifera* leaves. Each group of *M. oleifera* leaf extracts presented a dissimilar profile of the identified phenolic compounds, both in quantitative and qualitative terms. Therefore, it is difficult to point out the best extract preparation method to obtain a higher quantity of phenolic compounds. However, five of the identified compounds were present in all the prepared extracts, namely the compounds corresponding to the peaks **2**, **5**, **13**, **14**, and **18**. It is well supported by the literature that the extraction method (and solvent) used to recover phenolic compounds directly influences both the extraction yield and the phytochemical profile of the samples under analysis (Celep et al., 2019a), which explains the quantitative differences observed in this study.

The hydroethanolic extract of the wild *M. oleifera* leaf sample showed the main total concentration of phenolic compounds (103.7 ± 0.2 mg/g of extract), followed by the infused (51.8 ± 0.5 mg/g of extract) and decocted (47 ± 1 mg/g of extract) extracts of the same plant material, which can be explained by the use of different extraction solvents (ethanol and water) or by the application of heat in the preparation of the aqueous extracts (Celep et al., 2019b). On the other hand, the same extraction trend was not observed in the commercially acquired sample, since the higher concentration of phenolic compounds (72 ± 1 mg/g of extract) was found in the decocted extract, followed by hydroethanolic (65.3 ± 0.3 mg/g of extract) and infused (52 ± 1 mg/g of extract) extracts. This contrast in the total content of phenolic compound in the prepared extracts, and between wild collected and commercial acquired samples may be attributed to the processing and preparation methods already mentioned above to which the samples were subjected (Dias et al., 2016).

Despite the different phenolic profiles found between each of the extracts and the wild and commercial *M. oleifera* leaf samples, the group of flavonoids stands out in both cases, which is in agreement with the study of Ziani et al. (2019), whose investigation exposes a total concentration of flavonoids of up to 30 mg/g extract.

3.3. Biological activities of M. oleifera leaf extracts

Wild and commercial *M. oleifera* leaf hydroethanolic, infused, and decocted extract were tested for their capacity to prevent lipid peroxidation, using porcine brain cell tissues as oxidizable substrates, and the oxidative haemolysis using sheep RBC as a model. The results of both *in vitro* assays are presented in Table 5. Regarding the TBARS assay, the hydroethanolic extract of both wild and commercial samples showed the best antioxidant activity (IC_{50} values of 54 and 57 μg/mL) when compared with the aqueous extracts, and with no statistical difference between values. The lowest TBARS formation inhibition capacity (higher IC_{50} values) was achieved with the extract prepared by decoction, especially from leaves collected in the wild. The different solvents, temperatures, and extraction times may justify these differences, since certain antioxidant compounds may have been better extracted or degraded by these processing variables.

A different trend was observed in the OxHLIA assay (Table 5); the

Table 4
Content (mg/g of extract) of the phenolic compounds identified in the hydroethanolic, infused and decocted *M. oleifera* leaf extracts.

Peak	Wild sample extracts			Commercial sample extracts		
	Hydroethanolic	Infused	Decocted	Hydroethanolic	Infused	Decocted
1	2.6 ± 0.1	nd	nd	nd	nd	nd
2	5.5 ± 0.1^{e}	4.59 ± 0.01^{d}	6.07 ± 0.09^{b}	7.4 ± 0.1^{a}	5.8 ± 0.4^{c}	5.9 ± 0.3^{c}
3	5.49 ± 0.05^{d}	2.06 ± 0.02^{e}	nd	5.9 ± 0.2^{c}	6.2 ± 0.3^{b}	12.1 ± 0.3^{a}
4	1.31 ± 0.03^{c}	nd	2.21 ± 0.08^{b}	1.23 ± 0.03^{d}	2.97 ± 0.04^{a}	nd
5	5.6 ± 0.2^{c}	5.75 ± 0.09^{c}	4.7 ± 0.3^{d}	4.1 ± 0.3^{e}	6.16 ± 0.08^{b}	8.3 ± 0.4^{a}
6	nd	nd	nd	2.51 ± 0.08^{b}	nd	7.3 ± 0.4^{a}
7	8.38 ± 0.41^{b}	9.9 ± 0.5^{a}	nd	3.3 ± 0.1^{e}	5.8 ± 0.1^{c}	4.25 ± 0.04^{d}
8	13.8 ± 0.1^{a}	3.98 ± 0.04^{c}	9.5 ± 0.4^{b}	2.7 ± 0.1^{d}	nd	8.7 ± 0.2^{c}
9	7.49 ± 0.05^{a}	nd	4.1 ± 0.2^{c}	3.47 ± 0.05^{d}	nd	4.6 ± 0.2^{b}
10	4.98 ± 0.20	nd	nd	nd	nd	nd
11	1.26 ± 0.07	nd	nd	nd	nd	nd
12	6.27 ± 0.05^{a}	2.4 ± 0.1^{b}	nd	1.7 ± 0.1^{c}	nd	nd
13	13.7 ± 0.3^{a}	6.94 ± 0.08^{e}	10.5 ± 0.5^{c}	11.6 ± 0.5^{b}	8.0 ± 0.3^{d}	7.78 ± 0.07^{d}
14	5.4 ± 0.3^{a}	4.2 ± 0.2^{b}	3.33 ± 0.07^{d}	3.9 ± 0.2^{c}	5.33 ± 0.08^{a}	3.95 ± 0.02^{c}
15	7.1 ± 0.2^{a}	3.1 ± 0.1^{b}	1.27 ± 0.07^{d}	nd	1.91 ± 0.03^{c}	nd
16	nd	nd	nd	3.2 ± 0.1	nd	nd
17	nd	3.6 ± 0.2^{d}	nd	7.9 ± 0.9^{a}	6.3 ± 0.2^{b}	4.20 ± 0.09^{c}
18	6.7 ± 0.2^{a}	1.59 ± 0.06^{d}	2.3 ± 0.1^{c}	2.62 ± 0.05^{b}	1.66 ± 0.09^{d}	2.42 ± 0.03^{c}
19	2.8 ± 0.1^{a}	1.36 ± 0.05^{c}	1.61 ± 0.04^{b}	1.26 ± 0.06^{d}	nd	nd
20	1.66 ± 0.09^{a}	nd	nd	0.46 ± 0.01^{b}	nd	nd
21	1.23 ± 0.02^{a}	nd	nd	1.09 ± 0.05^{b}	nd	0.46 ± 0.01^{c}
22	nd	nd	nd	nd	nd	1.39 ± 0.05
23	1.41 ± 0.03^{a}	1.11 ± 0.03^{c}	0.81 ± 0.02^{e}	nd	1.23 ± 0.03^{b}	1.03 ± 0.02^{d}
24	nd	nd	nd	nd	0.94 ± 0.02	nd
25	1.11 ± 0.05^{a}	1.07 ± 0.05^{b}	0.91 ± 0.02^{c}	0.93 ± 0.04^{c}	nd	nd
TPA	20.6 ± 0.3^{c}	12.41 ± 0.08^{e}	13±1^{d}	21.1 ± 0.1^{b}	21.1 ± 0.5^{b}	33.5 ± 0.7^{a}
TF	83.15 ± 0.07^{a}	39.4 ± 0.6^{c}	34±1^{e}	44.2 ± 0.4^{b}	31.1 ± 0.5^{f}	38.7 ± 0.3^{d}
TPC	103.7 ± 0.2^{a}	51.8 ± 0.5^{d}	47±1^{e}	65.3 ± 0.3^{c}	52±1^{d}	72±1^{b}

Results are expressed mean ± SD ($n = 3$); nd: not detected: TPA: total phenolic acids; TF: total flavonoids; TPC: total phenolic compounds. Different letters in the same line indicate significant differences according to a Tukey's HSD test ($p < 0.05$). The peak identification is provided in Table 3. Calibration curves used in the quantification: Standard calibration curves: caffeic acid (y = 388345x + 406369, $R^2 = 0.999$, limit of detection (LOD) = 0.78 μg/mL and limit of quantitation (LOQ) = 1.97 μg/mL, peaks 1); clorogenic acid (y = 168823x - 161172, $R^2 = 0.999$, LOD = 0.20 μg/mL and LOQ = 0.68 μg/mL, peaks 2, 3, 5 and 6); *p*-coumaric acid (y = 301950x + 6966.7, $R^2 = 0.9999$, LOD = 0.68 μg/mL and LOQ = 1.61 μg/mL, peaks 4); apigenin-6-*C*-glucoside (y = 107025x + 61531, $R^2 = 0.9989$, LOD = 0.19 μg/mL and LOQ = 0.63 μg/mL, peaks 7, 8, 9, 10 and 12); quercetin-3-*O*-glucoside (y = 34843x – 160173, $R^2 = 0.9998$, LOD = 0.21 μg/mL and LOQ = 0.71 μg/mL, peaks 11, 16, 17, 18, 19, 20, 21, 22, 23, 24 and 25); quercetin-3-*O*-glucoside (y = 13343x + 76751, $R^2 = 0.9998$, LOD = 0.21 μg/mL and LOQ = 0.71 μg/mL, peaks 13, 14 and 15).

Table 5
Antioxidant activity of the hydroethanolic, infused and decocted *M. oleifera* leaf extracts.

Antioxidant assay[a]	Extract	Wild sample	Commercial sample	*p*-value
TBARS	Hydroethanolic	54 ± 3	57 ± 4	0.449
	Infused	318 ± 6	232 ± 4	<0.001
	Decocted	486 ± 8	417 ± 6	<0.001
OxHLIA, Δ*t* 60 min	Hydroethanolic	70 ± 2	81 ± 5	<0.001
	Infused	13 ± 1	1.8 ± 0.2	<0.001
	Decocted	14.5 ± 0.2	47 ± 2	<0.001
OxHLIA, Δ*t* 120 min	Hydroethanolic	na	na	–
	Infused	30 ± 2	6.1 ± 0.2	<0.001
	Decocted	49 ± 2	104 ± 3	<0.001

[a] Results are expressed as IC_{50} values (μg/mL); na: no activity; Trolox IC_{50} values: 23 ± 0.1 μg/mL (TBARS), 19.6 ± 0.8 μg/mL (OxHLIA, Δ*t* 60 min) and 41 ± 1 μg/mL (Δ*t* 120 min).

aqueous extracts were more effective in protecting 50 % of the RBC population form the AAPH-induced oxidative haemolysis. In fact, these extracts were more active than the synthetic antioxidant trolox used as a positive control, which is a very promising result for a natural plant extract. While the commercial leaf sample yielded lower IC_{50} values when prepared in infusion, the wild sample was more antioxidant when extracted by decoction. In addition, while both aqueous extracts exerted protective effects up to periods of 120 min, the hydroethanolic extract just protected the RBC for a 60 min Δ*t*.

The antioxidant activity of *M. oleifera* leaf extract has been reported by some authors. Chodur et al. (2018) evaluate the antioxidant activity of wild and domesticated *M. oleifera* samples and found that the last ones presented higher activity than the "wild type", which in agreement with the results herein obtained for the TBARS assay. An earlier report of Ilyas et al. (2015) also attributes a strong antioxidant activity to *M. oleifera* leaf powder when tested for DPPH radical scavenging activity.

The results of the antibacterial activity of the three *M. oleifera* leaf extracts are presented in Table 6. This activity was screened against six foodborne bacteria, namely *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Enterobacter cloacae*, and *Salmonella typhimurium*, against which strong inhibitory and bactericidal effects were observed, in most cases higher than the MIC and MBC values of the antibiotics streptomycin and ampicillin. The decoctions of leaves collected in the wild showed higher MIC and MBC values than the other two extracts (except for *E. cloacae*), thus requiring a higher extract concentration to inhibit or kill the tested bacterial strains. For commercial samples, infusions were the less effective against *S. aureus*, *E. cloacae*, *S. typhimurium*, yet the MIC/MBC values of these last two Gram-negative bacteria were lower or equal to those of the positive controls. Overall, wild samples appeared to have a better antibacterial capacity than the commercially acquired ones. A previous publication of Bukar et al. (2010) also describes that the *M. oleifera* ethanolic leaf extract exhibits a broad-spectrum activity against foodborne pathogens, such as *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, and *Enterobacter aerogenes*.

Table 6 also present the antifungal activity results achieved with the *M. oleifera* leaf extracts against *Aspergillus fumigatus*, *A. ochraceus*, *A. niger*, *Penicillium funiculosum*, *P. ochrochloron*, and *P. verrucosum* var. *cyclopium*. In general, the plant extracts exhibited strong inhibitory and fungicidal potential when compared to the positive controls ketoconazole and bifonazole. As observed for antibacterial activity, infusions

Table 6
Antibacterial (minimal inhibitory and minimal bactericidal concentrations (MIC and MBC, respectively) in mg/mL) and antifungal (MIC and minimal fungicidal concentration (MFC) in mg/mL) activity of the hydroethanolic, infused and decocted *M. oleifera* leaf extracts.

Microorganisms	Extract	Wild sample	Commercial sample	Streptomycin	Ampicillin
Bacterial strains		MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC
B. cereus	Hydroethanolic	0.037/0.15	0.075/0.15	0.04/0.1	0.25/0.45
	Infused	0.037/0.15	0.15/0.3		
	Decocted	0.15/0.3	0.075/0.15		
S. aureus	Hydroethanolic	0.1/0.15	0.15/0.3	0.1/0.2	0.25/0.4
	Infused	0.1/0.15	0.6/0.9		
	Decocted	0.3/0.60	0.15/0.3		
L. monocytogenes	Hydroethanolic	0.1/0.15	0.15/0.3	0.2/0.3	0.4/0.5
	Infused	0.1/0.15	0.15/0.3		
	Decocted	0.15/0.6	0.15/0.3		
E. coli	Hydroethanolic	0.05/0.075	0.075/0.15	0.2/0.3	0.4/0.5
	Infused	0.05/0.075	0.075/0.15		
	Decocted	0.15/0.3	0.15/0.3		
E. cloacae	Hydroethanolic	0.15/0.3	0.3/0.6	0.2/0.3	0.25/0.5
	Infused	0.1/0.3	0.6/0.9		
	Decocted	0.15/0.3	0.3/0.6		
S. typhimurium	Hydroethanolic	0.1/0.15	0.15/0.3	0.2/0.3	0.75/1.2
	Infused	0.1/0.15	0.2/0.3		
	Decocted	0.15/0.3	0.15/0.3		
				Ketoconazole	**Bifonazole**
Fungal strains		MIC/MFC	MIC/MFC	MIC/MFC	MIC/MFC
A. fumigatus	Hydroethanolic	0.075/0.15	0.075/0.15	0.25/0.5	0.15/0.2
	Infused	0.3/0.6	0.90/1.2		
	Decocted	0.075/0.15	0.075/0.15		
A. ochraceus	Hydroethanolic	0.037/0.075	0.037/0.075	0.2/0.5	0.10/0.2
	Infused	0.037/0.075	0.15/0.3		
	Decocted	0.037/0.075	0.037/0.075		
A. niger	Hydroethanolic	0.075/0.15	0.037/0.075	0.2/0.5	0.15/0.2
	Infused	0.075/0.15	0.075/0.15		
	Decocted	0.037/0.075	0.02/0.037		
P. funiculosum	Hydroethanolic	0.05/0.075	0.1/0.2	0.20/0.50	0.20/0.25
	Infused	0.05/0.075	0.6/0.9		
	Decocted	0.075/0.15	0.075/0.15		
P. ochrochloron	Hydroethanolic	0.15/0.3	0.075/0.3	2.5/3.5	0.20/0.25
	Infused	0.1/0.15	0.6/1.2		
	Decocted	0.15/0.3	0.075/0.3		
P.v. cyclopium	Hydroethanolic	0.075/0.15	0.075/0.3	0.2/0.3	0.1/0.2
	Infused	0.075/0.15	0.6/0.9		
	Decocted	0.075/0.15	0.075/0.3		

from the commercial sample were the least effective preparations against all tested fungi.

4. Conclusion

The present investigation emphasised the nutritional quality and bioactive potential of wild harvested and commercially acquired *M. oleifera* leaves. This funcional food is rich in carbohydrates (including sucrose, fructose, and trehalose), proteins, minerals, and PUFA (mainly C18:3n3 and C18:2n6), and vitamin E, due to the high contents of α-tocopherol. It also contains high levels of malic and citric acids, which may import certain organoleptic features, as well as ascorbic acid. The HPLC-DAD-ESI/MS^n analysis allowed identifying flavonoids (comprising apigenin, quercetin, isorhamnetin, and kaempferol glycoside derivatives) as the major class of polyphenols in hydroethanolic, infused, and decocted leaf extracts, followed by phenolic acids such as caffeoylquinic acids. The hydroethanolic and aqueous preparations were capable of inhibiting the TBARS formation and the oxidative haemolysis, and were effective against some food-borne bacterial and fungal strains.

The commercially acquired *M. oleifera* leaves show up with better quality composition than the wild collected ones, highlighting the importance of processing methods in the nutritional and chemical profile of this plant.

Altogether, these results highpoint the nutritional value of this low-cost functional food widely available in Guinea-Bissau and other tropical regions around the world, their suitability to fight malnutrition and increase food security in developing countries, and potential to be used in the development of nutraceutical formulations.

CRediT authorship contribution statement

Ângela Fernandes: Formal analysis, Methodology, Software, Validation, Investigation, Data curation, Writing - original draft. Ângela Liberal: Writing - original draft. José Pinela: Methodology, Software, Validation, Investigation, Methodology, Data curation, Writing - review & editing. Tiane C. Finimundy: Methodology, Software, Validation, Investigation, Data curation. Aducabe Bancessi: Investigation, Writing - review & editing. Ana Ćirić: Investigation, Formal analysis. Marina Soković: Investigation, Formal analysis; Luís Catarino: Supervision, Writing - review & editing. Isabel C.F.R. Ferreira: Supervision, Project administration. Lillian Barros: Conceptualization, Validation, Investigation, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

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